2011 Research Grant Program Winning Abstract

Influence of Soluble IL12Rβ1 on TH1/TH17 Differentiation

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The cytokines IL12, IL23, and IL12p40 homodimer influence a wide array of immune responses, impacting the development of both pathogen-driven immunity and autoimmunity. For these cytokines to mediate their effects, they must complex with IL12Rβ1, a receptor that spans the cell membrane and binds to the p40-domain of each cytokine. The subsequent association of the IL12Rβ1/cytokine complex with co-receptors IL12Rβ2 or IL23R confers cytokine specificity and initiates downstream signaling cascades. Over the course of investigating dendritic cells' (DCs) response to the pathogen *Mycobacterium tuberculosis*, we observed that DCs express—in addition to IL12Rβ1—an alternatively spliced isoform that (1) lacks the transmembrane (TM) domain and (2) contains an altered C-terminal amino acid sequence. Formation of this isoform (hereafter referred to as sIL12Rβ1) does not occur through enzymatic cleavage of surface IL12Rβ1 but rather through alternative splicing of the IL12rβ1 mRNA transcript. sIL12Rβ1 is secreted outside the cell (not yet published). Nevertheless it retains an ability to associate with the cell membrane and bind radiolabeled-IL12p40 despite lacking a TM domain (published). While we initially hypothesized that sIL12Rβ1 would function to "mop up" any IL12p40-containing cytokines and inhibit their function, in actuality we observed that sIL12Rβ1 enhances the effects of IL12p40-homodimer on DCs. The mechanism whereby sIL12Rβ1 affects IL12p40-homodimer binding is currently an area of investigation in my laboratory.

The aim of this BD Biosciences Immunology Research Grant is to determine whether the ability of sIL12Rβ1 to enhance signaling extends to the related cytokines IL12 and IL23. To determine this we will assay sIL12Rβ1's influence on a process that is extremely sensitive to IL12/IL23 signaling: the polarization of naïve $CD4^+$ T lymphocytes towards either a TH1 or TH17 phenotype. TH1 and TH17 differentiation are IL12rβ1 gene-dependent processes that are essential for protection and vaccination, respectively, against *M. tuberculosis* infection. These processes also contribute to organ-specific autoimmunity. To determine the influence of sIL12Rβ1 on T-cell polarization, we will be taking two experimental approaches that will rely upon BD technologies.

Experiment 1. Using fluorescently conjugated BD antibodies and a BD FACSAria™ cell sorter, we will purify naïve $CD4^+$ lymphocytes and subsequently stimulate them in the presence of anti-CD3/CD28 BD antibodies and recombinant BD cytokines (with or without purified sIL12Rβ1 that our lab has generated). The phenotype of these cells will then be assessed via fluorescence activated cell sorting analysis on a BD™ LSR II flow cytometer. Their cytokine-secretion pattern will be assessed using BD ELISA reagents and BD™ Cytometric Bead Arrays. Should differences be observed between groups, we would additionally use BD Phosflow™ reagents to examine whether the addition of sIL12Rβ1 alters cells' STAT phosphorylation patterns. As an alternative way to address the question of sIL12Rβ1's influence on mouse T-cell differentiation, we will transfect $CD90.2^+$ IL12rβ1KO T cells (which lack expression of both IL12Rβ1 and sIL12Rβ1) with cDNAs encoding either IL12Rβ1 alone, sIL12Rβ1 alone, or both IL12Rβ1 and sIL12Rβ1.

Transfection will be performed with recombinant lentivirus. The ability of transfectants (positively identified through co-transfection with CD90.1) to differentiate both in vitro and in vivo (following adoptive transfer into *M. tuberculosis*-infected animals) will then be assessed. This alternative approach would also be dependent upon BD fluorescent antibodies and analysis on the BD LSR II system.

Experiment 2. Given the influence of IL12 and IL23 on human health, modulating these cytokines' function is a goal of several experimental immunotherapies described in the published literature. Since we have discovered an endogenous modifier of mouse IL12p40 responsiveness, it is essential that we determine the relevance of our studies to human cytokine responses. For this, we will take a similar approach as described in Experiment 1, the difference being the usage of human PBMC-derived T cells (rather than mouse T cells) and the human homolog of sIL12Rβ1. Healthy donor T cells will be antiCD3/CD28 (BD) stimulated in the presence of BD recombinant human cytokines. BD antibodies against the surface antigens CD4 and CD45RA will be combined with intracellular staining reagents to assess the cytokine pattern of stimulated cells. Our hypothesis is that the distinct cytokine response pattern of mouse $CD4^+$ cells, stimulated in the presence of sIL12Rβ1, will be mirrored in human T cells. If this hypothesis is confirmed, these data will identify sIL12Rβ1 as a candidate for therapeutic manipulation (to attenuate autoimmunity) or as a part of a vaccination strategy (to promote pathogen-driven immunity).

Summary. Our preliminary data indicate that sIL12Rβ1 modulates cellular responses to IL12p40 homodimer. Whether this ability extends to other IL12-family members is the goal of this Immunology Research Grant. TH1/TH17 differentiation is the model system we will use to test this, as it is both (A) extremely sensitive to the modulation of either IL12/IL23 signaling and (B) easily assayed using an on-campus BD LSR II system and BD flow cytometry reagents, ELISA, and BD Cytometric Bead Arrays. The funds provided by this grant would also be used to determine whether our results using mouse cells are mirrored with human cells, allowing us to gauge the potential of our work to translate into human therapies.

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